

Amendments to the claims:

**Listing of claims:**<sup>1</sup>

1 (withdrawn). A method for detecting a target protein in a sample, comprising:

(a) capturing the target protein on an affinity capture probe;

(b) generating protein cleavage products of the target protein on the affinity capture probe using a proteolytic agent;

(c) detecting the protein cleavage products by laser desorption ionization mass spectrometry; and

(d) correlating one or more detected protein cleavage products with one or more prior-determined protein fragment markers of the target protein,

whereby the correlation detects the target protein.

2 (withdrawn). The method of claim 1 wherein the protein fragment markers are determined by:

(i) capturing the target protein on an affinity capture probe;

(ii) generating protein cleavage products on the affinity capture probe using a proteolytic agent;

(iii) analyzing at least one protein cleavage product with a tandem mass spectrometer, wherein analyzing comprises:

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<sup>1</sup> This listing of claims will replace all prior versions, and listings, of claims in the application.

(1) desorbing the protein cleavage products from the affinity capture probe into gas phase to generate corresponding parent peptide ions,

(2) selecting a parent peptide ion for subsequent fragmentation with a first mass spectrometer,

(3) fragmenting the selected parent peptide ion under selected fragmentation conditions in the gas phase to produce fragment ions, and

(4) generating a mass spectrum of the fragment ions with a second mass spectrometer; and

(iv) identifying at least one protein fragment marker of the test protein from among the candidate protein cleavage products by:

(1) submitting at least one mass spectrum to a protein database mining protocol which identifies at least one protein identity candidate for the test protein in the database based on a measure of closeness-of-fit between the mass spectrum and theoretical mass spectra of proteins in the database; and

(2) determining whether the identity candidate corresponds to the test protein;

whereby a correspondence indicates that the protein cleavage product is a protein fragment marker of the test protein.

3 (withdrawn). The method of claim 1 or claim 2 wherein mass spectrometry is laser desorption/ionization mass spectrometry.

4 (withdrawn). The method of claim 3 wherein mass spectrometry is laser desorption/ionization time-of-flight mass spectrometry.

5 (withdrawn). The method of claim 1 or 2 wherein the proteolytic agent is selected from the group consisting of chemical agents and enzymatic agents.

6 (currently amended). A method for identifying a protein that is differentially displayed between in the mass spectra of two complex biologic samples, comprising:

(a) with a mass spectrometer, detecting at least one protein that is differentially displayed between in the mass spectra of the two samples ~~with a mass spectrometer~~;

(b) ~~fragmenting~~ cleaving proteins in the two samples into protein cleavage products and with a mass spectrometer detecting protein cleavage products ~~fragments~~ that are differentially displayed in the mass spectra of ~~between~~ the two cleaved samples ~~with a mass spectrometer~~;

(c) determining the identity of at least one differentially displayed protein-~~fragment~~ cleavage product with a tandem mass spectrometer; and

(d) correlating the identity of the protein ~~fragment~~ cleavage product with a differentially displayed protein of step (a),

whereby the correlation identifies a differentially displayed protein.

7 (currently amended). The method of claim 6 wherein:

(a) detecting comprises:

(i) capturing proteins from the samples on affinity capture probes;

(ii) analyzing the captured proteins from each sample by laser desorption/ionization mass spectrometry;

(iii) comparing the mass spectra of the captured proteins in the two samples to identify proteins that are differentially ~~expressed~~ displayed;

(b) ~~fragmenting~~ cleaving and detecting comprises:

(i) capturing proteins from the samples on affinity capture probes;

(ii) generating protein cleavage products on the affinity capture probes using a proteolytic agent;

(iii) analyzing the protein cleavage products by laser desorption/ionization mass spectrometry;

(iv) comparing the mass spectra of the protein cleavage products in the two samples to identify protein cleavage products that are differentially ~~expressed~~ displayed; and

(c) determining the identity of at least one differentially displayed protein ~~fragment~~ cleavage product comprises:

(i) desorbing the protein cleavage products from the affinity capture probes ~~protein biochip~~ into gas phase to generate corresponding parent peptide ions,

(ii) with a first mass spectrometer, selecting a differentially displayed parent peptide ion for subsequent fragmentation ~~with a first mass spectrometer~~,

(iii) fragmenting the selected parent peptide ion under selected fragmentation conditions in the gas phase to produce product ion fragments with a second mass spectrometer,

(iv) generating a mass spectrum of the product ion fragments; and

(v) identifying at least one protein identity candidate for said differentially displayed cleavage product ~~fragment-marker products~~ by submitting at least one product ion mass spectrum to a protein database mining protocol which identifies at least one protein identity candidate for the differentially displayed protein cleavage product ~~in the database~~ based on a measure of closeness-of-fit between the product ion mass spectrum and theoretical mass spectra of proteins in the database.

8 (currently amended). The method of claim 6 wherein ~~fragmenting~~ cleaving is performed in solution.

9 (original). The method of claim 6 or 7 wherein the differentially displayed protein is detectable uniquely in one of said two samples.

10 (currently amended). The method of claim 6 or 7 wherein (b) ~~fragmenting~~ cleaving comprises enzymatic fragmentation.

11 (original). The method of claim 10 comprising limited enzymatic digestion.

12 (currently amended). The method of claim 6 or 7 wherein (b) ~~fragmenting~~ cleaving comprises chemical cleavage fragmentation.

13 (currently amended). The method of claim 12 wherein chemical ~~fragmentation~~ cleavage comprises acid hydrolysis.

14 (original). The method of claim 6 or 7 wherein the two samples are selected from (1) a sample from a healthy source and a sample from a diseased source, (2) a sample from a test model exposed to a toxic compound and a sample from a test model not exposed to the toxic compound or (3) a sample from a subject that responds to a drug and a sample from a subject that does not respond to the drug.

15 (withdrawn). A method for analyzing a protein analyte present as a plurality of cleavage products in admixture with cleavage products of other proteins, comprising:

(a) capturing a plurality of cleavage products from said mixture by adsorption to an affinity capture probe, said plurality of adsorbed cleavage products including at least one cleavage product of said protein analyte;

(b) washing said probe at least once with a first eluant for a time and under conditions sufficient to decrease the complexity of said plurality of adsorbed protein cleavage

products, said adsorbed cleavage products of reduced complexity including at least one cleavage product of said protein analyte; and then

(c) characterizing said at least one cleavage product of said protein analyte with a tandem mass spectrometer measurement,

said tandem mass spectrometric characterization of said at least one cleavage product providing an analysis of said protein analyte.

16 (withdrawn). The method of claim 15, further comprising the antecedent step of:

cleaving proteins in said mixture into cleavage products with a proteolytic agent.

17 (withdrawn). The method of claim 15 or claim 16, further comprising at least one iteration of the step, after washing with said first eluant and before characterizing said at least one protein analyte cleavage product, of:

washing said probe with a second eluant, said second eluant having at least one elution characteristic different from that of said first eluant, for a time and under conditions sufficient further to decrease the complexity of said plurality of adsorbed protein cleavage products, said adsorbed cleavage products of further reduced complexity including at least one cleavage product of said protein analyte.

18 (withdrawn). The method of claim 15, wherein said characterizing with a tandem mass spectrometer measurement comprises:

- i) desorbing and ionizing said protein cleavage products from said probe, generating corresponding parent peptide ions;
- ii) selecting a desired parent peptide ion in a first phase of mass spectrometry;
- iii) fragmenting said selected parent peptide ion in the gas phase into fragment ions; and
- iv) measuring the mass spectrum of the fragment ions of said selected parent peptide ion in a second phase of mass spectrometry.

19 (withdrawn). The method of claim 18, wherein said fragmenting is effected by collision induced dissociation (CID).

20 (withdrawn). The method of claim 19, further comprising:

- (d) determining at least a portion of the amino acid sequence of said protein analyte by calculating differences in masses among fragment ions represented in said fragment ion mass spectrum.

21 (withdrawn). The method of claim 20, further comprising:

- (e) determining at least one protein identity candidate for said protein analyte based upon the closeness-of-



fit calculated between said predicted sequence and sequences prior-accessioned into a sequence database.

22 (withdrawn). The method of claim 21, further comprising:

(f) assessing the likelihood that said identity candidate is the same as said protein analyte by comparing (i) the mass measured for said selected parent peptide ion to (ii) the masses predicted for cleavage products that would be generated by cleaving said identity candidate with said proteolytic agent,

a match as between a predicted mass and said measured mass indicating increased likelihood that said identity candidate is the same as said protein analyte.

23 (withdrawn). The method of claim 15, wherein said tandem mass spectrometric characterization is performed using a mass spectrometer selected from the group consisting of QqTOF mass spectrometer, ion trap mass spectrometer, ion trap time-of-flight (TOF) mass spectrometer, time-of-flight time-of-flight (TOF-TOF) mass spectrometer, and Fourier transform ion cyclotron resonance mass spectrometer.

24 (withdrawn). The method of claim 23, wherein said tandem mass spectrometer is a QqTOF mass spectrometer.

25 (withdrawn). The method of claim 15, wherein said affinity capture probe has a chromatographic adsorption surface.

26 (withdrawn). The method of claim 25, wherein said chromatographic adsorption surface is selected from the group consisting of reverse phase surface, anion exchange surface, cation exchange surface, immobilized metal affinity capture surface and mixed-mode surface.